

Evaluation of cleaning and disinfection procedures against *Salmonella enterica* at swine farms, transport and lairage facilities

Arguello, H.*

Rubio, P., Jaramillo, A., Barrios, V., García, M., Carvajal, A.

Infectious Diseases and Epidemiology, Faculty of Veterinary Medicine, University of Leon, Spain.

*corresponding author: hector.arguello@unileon.es

Abstract

Evaluation of the cleaning and disinfection protocols effectiveness against *Salmonella* in three points of the pork production chain: finishing farm, transport and lairage. A 22.2% of the farms, 62.5% of the slaughter trucks and 63.6% of the holding pens tested were positive to *Salmonella* after cleaning and disinfection procedures. The other samples collected in trucks and lairage shows that there is also contamination before the pigs staying. These results show that the protocols carried out at the farms, trucks and abattoirs included in this survey are not efficient to eliminate *Salmonella*.

Introduction

Salmonella enterica is one of the most common and widely distributed food-borne pathogens. The bacteria can contaminate almost any food type although raw eggs, poultry and pork are the most common sources of human outbreaks of salmonellosis [1]. Contamination can occur at any point of the food chain including primary production. Due to the ability of *Salmonella* to survive during large periods of time in the environment, particularly in the presence of organic matter, cleaning protocols are of outstanding importance. This investigation aimed to assert the efficacy of routinely cleaning and disinfection procedures performed at three points of the pork production chain: finishing farms, transport and lairage.

Material and Methods

a) Study design

Finishing farms: Thirty-six pig finishing farms performing a strict all-in/all-out management (AI/AO) were evaluated. Within each farm, twelve samples were collected from pen floors (5 samples), pen walls (5 samples), corridors (1 sample) and dust (1 sample) after cleaning and disinfection procedures, just before the entrance of a new batch of animals.

Transport: Eight slaughter trucks were evaluated in three sampling rounds: (1) before loading the animals at the farm (preload), (2) after unloading the pigs at the abattoir (postload) and (3) after cleaning and disinfection procedures. Each truck included three storeys and therefore three samples, one per floor, were collected in each sampling round. In total, nine samples were recovered from each monitored slaughter truck. Surface samples were collected at preload and after cleaning and disinfection procedures, while floor faeces were collected at postload.

Holding pens: Sixty-six holding pens in two different abattoirs were evaluated in four sampling rounds: (1) before the entry of the pigs, (2) at half of the working day, (3) at the end of the working day and (4) after cleaning procedures. Surface samples were collected in sampling rounds 1 and 3 while faecal samples were collected in the other two sampling rounds (2 and 3) during the staying of pigs.

b) Sample collection

Surface samples were collected using gauzes previously moistened in peptone buffered water (PBW). Each sample was obtained by swabbing five points of 25cm x 25cm.

Floor faecal samples were constituted by five fresh fecal pinches collected at five different locations of the pen or lorry.

c) Isolation methodology

Bacteriological analyses were made according to EN-ISO 6579:2002/Amd 1:2007. Gauzes immersed in 50 ml of PBW or pool faeces were sent to the laboratory immediately after their collection in cooling conditions. One isolate of each positive sample was further serotyped by slide agglutination according to Kaufmann-White scheme using commercial antisera.

Results

Eight farms (22.2%) had at least one *Salmonella* positive sample after cleaning and disinfection procedures (Table 1). *Salmonella* was recovered mainly from floor samples (6 out of 8 positive farms were positive in floor samples) followed by pen walls (three farms); it is remarkable that the contamination was only detected in corridors in two of the positive farms. In contrast, *Salmonella* was not isolated from dust samples in any of the farms included in the present study. *S. Typhimurium* was identified in seven out of the eight contaminated farms while *S. 4,[5],12:i:-* was detected in the remaining one. Two different serotypes, *S. Typhimurium* and *S. Rissen*, were recovered in one farm.

Results obtained in the evaluation of eight slaughter trucks are included in Table 2. Fifty percent of the samples and 50% of the trucks were positive before the loading of the pigs indicating that lorries were already contaminated by *Salmonella*. Contamination was also detected in 50% of the trucks after the staying of the pigs. Moreover, five of the trucks (62.5%) were positive after cleaning and disinfection procedures. Altogether, percentage of *Salmonella* positive samples was 26.1% before loading the pigs, 39.1% immediately after unloading and 34.8% after cleaning and disinfection procedures. Regarding the distribution of contamination within the three storeys of each truck, 12 positive samples were recovered from first floor (50%) and 7 (29%) and 6 (25%), from second and third respectively. Despite that the number of positive samples found in the first floor was twice the other two, no statistically significant differences were demonstrated ($\chi^2=3.8$, $p=0.14$).

A total of 66 holding pens were evaluated in a total of six different visits to two commercial pig slaughterhouses (Table 3). *Salmonella* contamination was detected in 39.4 % of the samples collected before the entry of the pigs, 98.5% of the samples collected at half of the working day and 80.3% of the samples collected at the end of the working day. Moreover, *Salmonella* was recovered in 42 out of the 66 holding pens (63.6%) after cleaning procedures.

Discussion

Herein we present the results of a brief research to determine the effectiveness of the routinely cleaning and disinfection protocols carried out at three different stages of the pork production chain in Spain: farm, transport and lairage. Although our results were obtained from a limited number of farms, slaughter trucks and holding pens, they clearly show that *Salmonella* can persist in the environment after routinely cleaning and disinfection procedures at these three evaluated points. Taking into account that *Salmonella* is sensitive to the most commonly used disinfectants, our results indicate that cleaning and disinfection procedures were not performed properly.

At the farm level, strict all-in/all-out management has been pointed as a proper control measure to avoid transmission of the bacteria between batches of animals. However, according to our results even when cleaning procedures were classified as satisfactory by clinicians and a phenol derivate disinfectant was used, *Salmonella* was still detected in one of each five investigated farms. It has been described that holes in floors and walls make difficult the penetration of disinfectant solutions and what is more, the biofilms created by *Salmonella* can make the action of the disinfectants difficult [2]. Moreover, particular attention should be paid not only to pens but also to corridors in order to prevent infections between batches.

In a similar way, *Salmonella* was recovered from fifty percent of the evaluated slaughter trucks before the loading of the pigs at the farm. The fact that more than sixty percent of the trucks sampled after cleaning and disinfection procedures gave at least one positive sample reveals the ineffectiveness of these procedures. A similar result was obtained in the evaluation of holding pens in slaughterhouses. *Salmonella* was recovered in more than 60% of the samples collected immediately after cleaning and disinfection procedures and in almost 40% of the floor swabs collected at the start of the working day, before the entrance of the pigs. These results are in agreement with previous researches in both slaughter trucks [3] and lairage of pig slaughterhouses [4,5]. Taking into account the short period of time needed by *Salmonella* to reach and multiply in the intestine of susceptible pigs [5], contamination at both levels is relevant and would explain at least part of the new infections occurring between the farm and the slaughterhouse.

Conclusion

Present research demonstrates that routinely cleaning and disinfection procedures performed at the farm level as well as in the slaughter trucks and the lairage of pig slaughterhouses are not able to eliminate *Salmonella* properly. According to this, slaughter trucks and holding pens should be taken into consideration as sources of new infections in pigs entering the slaughterhouse. An effort to improve these cleaning and disinfection protocols have to be done as part of the *Salmonella* control strategies at the slaughter level. On the other hand, cleaning and disinfection procedures should also be improved in pig farms performing all-in/all-out management in order to avoid cross-contamination between batches of animals.

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Table 1. Distribution of Salmonella positive samples in the eight pig finishing farms where Salmonella contamination was demonstrated after cleaning and disinfection procedures. A total of 36 pig farms performing strict all-in/all-out management were evaluated.

	Farm 1	Farm 2	Farm 3	Farm 4	Farm 5	Farm 6	Farm 7	Farm 8
Pen Floor ¹	1	5	1	0	0	3	3	2
Pen Walls ¹	0	2	2	0	0	0	1	0
Corridors ²	0	1	0	1	1	0	0	0
Dust ²	0	0	0	0	0	0	0	0
Total	1	8	3	1	1	3	4	2

¹ Five samples were collected in each farm

² One sample was collected in each farm

Table 2. Distribution of Salmonella positive samples in eight slaughter trucks that were evaluated in three sampling rounds: before loading the animals at the farm, after unloading the pigs at the abattoir and after cleaning and disinfection procedures. Within each sampling round, three samples were collected in each of the three storeys of the truck.

	Before loading pigs			After unloading pigs			After cleaning and disinfection procedures		
	First floor	Second floor	Third floor	First floor	Second floor	Third floor	First floor	Second floor	Third floor
Truck 1	+	+	-	+	+	-	+	+	-
Truck 2	-	-	-	-	-	-	-	-	-
Truck 3	-	-	-	+	-	+	-	-	-
Truck 4	-	+	-	+	+	+	+	+	+
Truck 5	-	-	-	+	-	+	+	-	-
Truck 6	+	+	-	-	-	-	+	-	-
Truck 7	+	-	-	-	-	-	+	-	-
Truck 8	-	-	-	-	-	-	-	-	-

+ Salmonella positive sample; - Salmonella negative sample

Table 3. Prevalence of Salmonella contamination in sixty-six holding pens of two commercial slaughterhouses (slaughterhouse A and slaughterhouse B). Each slaughterhouse was visited in three different days (visit I to visit III). Each sampling day, a total of four sampling rounds were performed in each holding pen: before the pig entry, at half of the working day, at the end of the workind day and after cleaning procedures.

	Slaughterhouse A			Slaughterhouse B			TOTAL
	Visit I	Visit II	Visit III	Visit I	Visit II	Visit III	
Before pigs entry	0%	36.4%	54.5%	100%	27.3%	18.2%	39.4%
Half working day	45.5%	72.7%	81.8%	100%	100%	100%	98.5%
End of working day	18.1%	81.8%	90.1%	100%	90.1%	100%	80.3%
After cleaning	27.3%	54.5%	54.5%	90.1%	81.8%	72.7%	63.7%